Development of treatment strategies to combat Ebola and Marburg viruses

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Ebola and Marburg viruses are emerging/re-emerging pathogens that pose a significant threat to human health. These naturally occurring viral infections frequently cause a lethal hemorrhagic fever in humans and nonhuman primates. The disastrous consequences of infection with these viruses have been pursued as potential biological weapons. To date, there are no therapeutic options available for the prophylaxis or treatment of infected individuals. The recognition that Ebola and Marburg viruses may be exploited as biological weapons has resulted in major efforts to develop modalities to counter infection. In this review, select technologies and approaches will be highlighted as part of the critical path for the development of therapeutics to ameliorate the invariably devastating outcomes of human filoviral infections.

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Ebola and Marburg virus, family Filoviridae, are among the most lethal microbes known to humans, with case fatality rates often approaching 90%. Since their discovery several decades ago, episodes or outbreaks have been sporadic and largely contained in endemic areas, primarily in Central Africa. However, concern about the natural or unnatural introduction of these agents outside of the endemic areas has dramatically increased both research interest and public awareness of the filoviruses. Currently, there are no preventive vaccines or postexposure treatments available for human use. However, significant progress has been made over the last 5 years in developing preventive vaccines against the filoviruses. For example, a candidate vaccine based on a recombinant replication-defective adenovirus completely protected nonhuman primates (NHPs) from Ebola virus infection [1,2], while a second candidate vaccine based on a recombinant replication-competent vesicular stomatitis virus completely protected monkeys against both Ebola and Marburg viruses [3]. Progress in developing treatments and therapies against the filoviruses has been much slower, and no postexposure modality has been able to uniformly protect NHPs.

Horizons for filovirus therapeutics

The lack of therapeutics targeting filoviruses is an important public health concern [4]. The field can be segregated into:

- Molecules targeting the interplay between the viral machinery and its host
- The viral machinery itself
- Inducers of an inhospitable host
- Compounds that lessen the impacts of the disease

Naturally occurring filovirus transmission between humans can be constrained through the use of barrier nursing practices [5–7]. In spite of this, aspects of controlling outbreaks in epidemic regions and facets of biological weapons mandate the development of prophylaxis as additional therapeutic options [4].

On the course to the development of filoviral therapeutics, there are a few key advances. Notably, the development of reverse genetics systems [8–11], NHP [12–15] and rodent model systems [16,17] and an improved understanding of the clinical picture of filoviral hemorrhagic fever (HF) [18–24] provide important foundational elements toward successful drug discovery. The reverse genetics systems have provided the ability to genetically manipulate the virus.

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14. ABSTRACT

Ebola and Marburg viruses are emerging/re-emerging pathogens that pose a significant threat to human health. These naturally occurring viral infections frequently cause a lethal hemorrhagic fever in humans and nonhuman primates. The disastrous consequences of infection with these viruses have been pursued as potential biological weapons. To date, there are no therapeutic options available for the prophylaxis or treatment of infected individuals. The recognition that Ebola and Marburg viruses may be exploited as biological weapons has resulted in major efforts to develop modalities to counter infection. In this review, select technologies and approaches will be highlighted as part of the critical path for the development of therapeutics to ameliorate the invariably devastating outcomes of human filoviral infections.

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For drug-discovery efforts, this has culminated in the development of an engineered Ebola virus expressing the green fluorescent protein (GFP) [9]. GFP is expressed as an extra transcriptional unit. The resulting fluorescent signal is dependent upon the activity of the RNA-dependent RNA viral polymerase. Using this recombinant virus, a high-throughput cell-based assay was developed.

For safety and security reasons, filoviruses can only be investigated in Biosafety Level (BSL)-4 laboratories. This has been one of the traditional impediments to drug discovery. The new fluorescent cell-based, drug-discovery assays are facilitating the higher throughput screening of chemical libraries. This assay has a shortened incubation time, reduced manipulations and a larger linear range. Previous library screening efforts were based on the use of surrogate viruses such as respiratory syncytial virus as a predictor of potential antivirals. To date, this approach has not been successful. It is not surprising given the significant sequence variation between the different viral families.

Animal models are critical for the initial evaluation of efficacy, subsequent development of dose, schedule and regimen, and more importantly, a convenient system to assess possible intervention points. Currently, there are NHP models for Marburg and Ebola viruses [13,15], a guinea-pig model for Marburg and Ebola virus [17] and a mouse model for Ebola virus [16]. The NHP models provide the best recapitulation of human disease, while the rodent models have significant gaps, particularly in observed pathology compared with primates [25], although they are more convenient. Rodent model utility is best restricted to evaluating antivirals that target the viral machinery. However, the NHP models for Ebola virus have proven to be invaluable tools. In fact, a temporal study using a cynomolgus macaque model has provided valuable insight into the pathology and pathogenesis associated with Ebola virus infection [12,14]. The enhanced understanding of the dysregulation of the coagulation system has led to the first treatment modality with efficacy in a NHP model system [26]. By interfering with the tissue-factor pathway of the coagulation cascade, some animals were able to survive infection. The enhanced understanding of the pathophysiology may lead to other weak points in the viral replication strategy. This understanding of the multisystem response to infection has led to a potential new class of inhibitors that target the disease process rather than the virus itself.

The pathogenesis of filoviral infection of humans is substantially lacking. The difficulty in documenting the natural history of filoviral infection of humans has been, in part, due to the distance of human cases from contemporary medical care and laboratory facilities and the need for high-level containment. However, a few studies have been performed under field conditions, which included portable and/or improvised laboratories for evaluating archived samples sent to high-containment facilities outside of endemic areas. A recent report suggested that lower peak viral loads correlated with a better outcome [24]; thus, a potential antiviral may not need a sterilizing effect to transform Ebola virus infection into a more survivable disease. This finding is consistent with the improved outcomes of HIV-infected patients with reduced viral loads. Natural history studies are needed to improve our understanding of the clinical picture of disease. These studies will lead to developing correlates of survival. The critical parameters that tip the balance between surviving or succumbing to a filoviral infection need to be further clarified. Critical parameters may influence future drug design.

Together, these advances are providing the foundation for a successful drug discovery leading to an effective antiviral. However, the successful identification of compounds and drugs that inhibit viral replication lies with traditional issues of pharmacokinetics, bioavailability, drugability and toxicity. The conversion of a drug hit to an effective antiviral will depend on the successful resolution of these issues. These aspects of drug development are often not given the appropriate attention by investigators. Translation of *in vitro* antiviral activity to *in vivo* efficacy may be lost owing to poor pharmokinetics, toxicity and bioavailability. Advancing a promising *in vitro* led to *in vivo* efficacy should include considerations of pharmokinetics, toxicity and bioavailability.

Biology of filoviruses

Filoviruses are negative-sense, single-strand, enveloped viruses (biology reviewed in [27–31]). The unique ultrastructural appearance of the virion is the basis of the name (greek = thread like). The virus is composed of two major components. The viral envelope is composed of host-derived plasma membrane studded with a virally encoded type 1 glycoprotein (GP). Likely, the VP24 protein is also part of the envelope. Contained within the virion is the ribonucleoprotein (RNP). The RNP is built around the negative-strand RNA genome encased with the nucleoprotein (NP) and the minor nucleoprotein (VP30). The viral polymerase complex is composed of the large (L) protein and a phosphoprotein (VP35). Filovirus entry is mediated by attachment to cell-surface receptors through the viral GP. The virus is internalized into the cellular endosomes. The acid pH of the endosome is thought to trigger a confirmation shift of the GP protein to zipper the coiled-coil domains together, bringing the viral membrane and cellular membrane into close proximity. The two membranes fuse releasing the RNP into the cytosol.

In the cytosol, the RNP can initiate the transcriptional program. The RNA-dependent RNA polymerase transcribes each gene. At some point during transcription, a switch is initiated, and the polymerase will begin to replicate the genome. Genomic RNA is encapsidated while messenger transcripts are not. The entire replicative cycle occurs in the cytosol of the cell. Assembly is believed to be orchestrated by VP40. VP40 is thought to function as a 'bandleader'. Nascent RNP structures are assembled and brought to the plasma membrane by interaction with VP40 along rails of actin. At the plasma membrane, the RNP meets with GP that has been processed and transported to the cell surface through the

endoplasmic reticulum–Golgi-body complex. At the cell surface, the RNP buds through the plasma membrane capturing the viral envelope. The forming viral membrane fuses with itself and pinches off from the cell. This particle is ready to infect another cell.

Pathology & pathogenesis of filoviruses

Identifying early and strategic events that lead to the development of severe filoviral HF is important for the rational development of treatments and therapies. As noted before, few cases of filoviral HF have been managed and studied outside of the endemic region of Central Africa. Nonetheless, a handful of studies have provided valuable information on the kinetics of the host immune response to filoviral infection. In addition, animal models, in particular NHP, have provided important information regarding the pathogenesis of filoviral HF.

Ebola and Marburg infection of humans and NHP are characterized by coagulation disorders, by a dramatic loss of lymphocytes and a concomitant degeneration of lymphoid tissues. It is important to understand, however, that lymphocytes do not support the replication of Ebola or Marburg virus. The massive die-off of lymphocytes during the course of infection occurs by the process of apoptosis [32,33], although the mechanism for this 'bystander' loss of lymphocytes is unknown.

Filoviral infection appears to trigger a strong proinflammatory response both *in vitro* and *in vivo* as reported in a number of studies [12,26,34–39]. Across these studies, several inflammatory mediators including interleukin (IL)-6, tumor necrosis factor (TNF)-α and nitric oxide (NO) appear to be consistently implicated as playing an important role in the development of filoviral HF. Specifically, TNF-α and NO are thought to contribute to the vascular instability seen during filoviral infection [39–41] and in one study, elevated blood levels of NO were in fact associated with mortality [38].

Although defects in blood coagulation and fibrinolysis are consistent features of filoviral HF, the loss of blood is infrequent and, in fact, even when present, is not significant enough to account for death. Disseminated intravascular coagulation (DIC) is often viewed as a notable manifestation of filoviral HF. A number of studies have shown histological and biochemical evidence of DIC syndrome during filoviral infection of humans and NHP [12,14,25,26,42–48]. The mechanism(s) for triggering the coagulation disorders is not completely understood. For Ebola HF, recent studies have proposed that the coagulopathy is triggered by several factors, particularly during later stages of disease, and implicate the expression/release of tissue factor from filovirus-infected monocytes/macrophages as a major factor in inducing the coagulation defects observed in filoviral infections [14].

Inhibitors of the viral machinery

An attractive target for antivirals is to disrupt the viral machinery. Filoviruses are economical viruses encoding seven viral proteins. In comparison, orthopoxviruses require almost 200 proteins to successfully perform the viral replication program. Currently, all of the Ebola virus proteins are believed to be essential for competent replication. Some of the more promising potential inhibitors of the viral machinery are targeting membrane fusion, viral replication/transcription and assembly.

Membrane fusion

Filovirus entry is a multistep process. The viral genomic material (RNP) is delivered to the cytosol through the fusion of the viral and cellular membranes constructing an exit for the RNP from the virion. Delivered to the cytosol, the RNP can initiate the viral transcription and replication program. The attachment and fusion mechanisms are mediated by the viral GP. GP is a disulfide-linked heterodimer of GP1 and GP2 [49–54].

GP2 is presumably responsible for the viral fusion [53,55–62]. The GP2 subunit contains structural similarities to other class I membrane proteins such as the influenza virus HA1 and HIV gp41 proteins. Enfuvirtide (T20) has shown efficacy for treating HIV infection by specifically targeting the coiled-coil motif in gp41 [63]. T20 is a competitive inhibitor of the coiled-coil motif in gp41. The coiled-coil domain of the Ebola virus GP2 is a promising target [55–57,60,61,64]. This domain has significant structural homology to the established motifs in the influenza virus HA1 and HIV gp41 [53,64,65]. Several groups have shown that peptides can competitively inhibit this fusion activity [61]. Genetic studies have shown that with pseudotyped viruses packaging mutant GP molecules, the predicted coiled-coil domain is essential for GP function [55–57,64]. Inhibiting the coiled-coil motif may prevent the release of the RNP into the cytosol. The motif is highly conserved between Ebola and Marburg viruses. It is possible that a single small molecule could target both, but it is likely that unique molecules would be needed.

Another approach to interfering with filoviral fusion was recently raised by studies performed by Chandran and colleagues [66]. This group proposed that the conformational changes that occur in the Ebola virus GP that initiates fusion, requires the activity of the endosomal cysteine proteases, cathepsin B and cathepsin L. This group further reported that inhibitors of these cathepsins reduced the production of Ebola virus *in vitro*, and suggested that these compounds may have therapeutic utility. It has been noted that these compounds are not likely to be used *in vivo* as they are toxic to cells, but that development of less toxic cathepsin inhibitors warrants investigation [67].

Viral replication/transcription

Viral genomic transcription and replication are highly drugable targets. Several successful antivirals have targeted genomic transcription/replication, for example, the nucleoside inhibitors of HIV, acyclovir for herpes simplex virus and ribavirin for the arenviruses and bunyaviruses. Filoviruses replicate and transcribe their genomes using a virally encoded RNA-dependent RNA polyermase. This polymerase complex is composed of the NP, VP35, VP30, the L protein and a RNA genome [68]. To date, there are no compounds specifically

targeted to this complex. However, this target is highly amenable to drug discovery. The replication transcription machinery can be reconstituted *in vitro* using recombinant plasmids expressing the four proteins and a synthetic model genome encoding a marker protein (minigenome system) [69]. The marker gene encoded by the model RNA will only be transcribed if a functional RNA polymerase is reconstituted. Most importantly, this system would not produce infectious viruses. The minigenome system is amenable to high-throughput drug discovery in a BSL-2 environment. Large chemical libraries can be down-selected and a smaller subset of compounds tested using infectious viruses in the BSL-4 facilities.

This minigenome system is also amenable to using a peptidomics approach for discovery of rationally designed inhibitors. Compounds could be targeted to disrupt RNA binding and viral protein/protein interactions. The association of the NP, VP30, VP35, L and RNA are essential to perform the replication/transcription program [69]. VP30 oligomerizes and is essential for replication [70]. Oligopeptides that disrupt the oligomerization are able to reduce the percentage of infected cells. The concentrations needed to achieve inhibition are higher than therapeutically applicable; it would be possible to mine small molecules with similar activities with the expectation of developing a compound that could be evaluated *in vivo*. However, many of the other essential interactions have not been extensively mapped. It is possible to determine the interactions through various biochemistry and genetics-based systems.

A third target may be through the use of nucleoside analogs to inhibit chain elongation or create an error catastrophe event. Error catastrophe may be the mechanism behind the inhibition of Hantaan virus by ribavirin [71]. Ribavirin, the prototypical inhibitor for many RNA viruses is not effective against Ebola [72] or Marburg virus [73]. However, there are many derivatives of nucleoside analogs generated for HIV drug discovery and anticancer discovery efforts that may contain activity that have yet to be tested. These compounds would be amenable to screening through the minigenome system, and the subsequent down-selected compounds would be evaluated by using the fluorescent cell-based assay.

A relatively new technology is the use of RNA interference (RNAi). RNAi is a nucleic acid-specific method to ablate intracellular RNA molecules. There are several examples of RNAi technology being used as antivirals to treat viral infections, including Marburg virus [74], in cell culture experiments. Experience from animal models suggests that this technology may have significant *in vivo* potential [75]. The molecules are delivered as RNA oligos, DNA constructs expressing the RNAi molecule and defective retroviruses. The difficult factor is the choice of target sequence and delivery. Targets in the genome would be difficult to achieve. The filoviral genomes are encapsidated with a nucleocapsid protein protecting the RNA. However, viral messenger transcripts are unprotected by the nucleocapsid. It is believed that the abundance of messenger RNA (mRNA) correlates with the position of the gene relative to the 3´ end. Like all single-strand RNA, there will be regions that are structured and inaccessible to the RNAi molecule. There is a greater abundance of NP transcripts than VP35, and many more than L using the vesicular stomatitis virus model. It may be easier to target lower abundance transcripts that produce essential genes than higher abundance molecules. Furthermore, disrupting the ratio of gene transcripts negatively affects the fitness of the virus. Complete ablation of mRNA transcripts may not be necessary to significantly affect the outcomes of filoviral infections. Filoviruses have significant sequence variability. However, they are hyperconserved domains which may serve as useful targets.

Inhibitors of the viral machinery & the host

Filoviruses corrupt cellular machinery to transform the environment into a favorable cellular condition for replication and to perform the replication program. Many of the viral proteins interact with host cellular proteins. These protein/protein interactions are often essential for viral replication. There are several interesting virus host protein–protein interactions that if disrupted, may yield an effective antiviral.

VP40 interacts with several host proteins to promote the viral budding process [76–81]. These interactions are mapped to a proline-rich motif known to interact with cellular proteins containing the WW domains [80]. Recent studies have shown that this motif is not essential for viral replication. With reverse genetics, the PPxY motif was genetically ablated, which resulted in peak viral yields reduced by 1 log [82]. However, as noted previously, Towner and colleagues found that in Ebola virus-infected patients, reduction of viral loads correlated with improved outcomes [24]. As a result, it may not be necessary to achieve a sterilizing effect to achieve an effective antiviral.

VP35 functions as the viral interferon (IFN) antagonist [83]. The host protein IFN regulatory factor (IRF)-3 is a major effector of the cellular innate immune response. VP35 inhibits the activation of IRF-3 and the subsequent downstream events [84]. It is also thought that VP24 may function, in part, as an IFN antagonist [85]. The innate immune response most likely has a pivotal role in productive infection of Ebola virus. Viral IFN antagonists modulate the host by releasing the IFN-induced blocks to replication. Viruses lacking IFN antagonists are often restricted to replicating in IFN-deficient hosts. By targeting the activity of VP35 and/or VP24, Ebola virus may be more susceptible to the antiviral activities of the innate immune system [86].

GP functions to attach viral particles to cell-surface receptors [87,88]. There has been considerable effort in the development of monoclonal antibodies for treating filoviral HF [89–92]. Passive transfer experiments have been highly successful in rodent models, but these encouraging results have unfortunately not translated as well to NHP. For example, high-titer hyperimmune horse serum failed to protect NHP when administered immediately after Ebola virus infection [93]. Convalescence whole blood from Ebola-immune rhesus monkeys also failed to protect treated macaques from an experimental Ebola virus challenge (TW GEISBERT, UNPUBLISHED OBSERVATION).

Conversely, a few studies reported beneficial effects in NHP using Ebola-specific immune globulins [94,95]. Differences in NHP species and challenge doses used may account for the different outcomes among these various passive immunotherapy efforts.

Isolating the cell-surface receptor for filoviruses may provide additional targets. Filoviruses use cell-surface receptors to gain cellular entry mediated by the viral GP. GP has been a traditional target for antibody-based strategies. Small molecules were recently shown to be effective at blocking viral entry for HIV. BMS-806 was discovered through a cell-based entry-screening assay [96]. Although the compound does not interfere with viral attachment, it prevents coiled-coil domains from being exposed independent to the activity of T20. Similar approaches could be developed for filoviruses. A similar compound for filoviruses would likely have to be stably bound through a large pH range. GP pseudotyped viral vectors expressing various reporter genes could be used for drug-discovery screening. Wool-Lewis and Bates demonstrated that GP could be pseudotyped with the murine leukemia virus retroviral core [88]. The pseudotyped constructs were neutralized by Ebola virus-specific antibodies and entered the cell through the endosomal pathway. These types of surrogate reporters for filoviruses could be used to screen large chemical libraries for entry inhibitors. GP pseudotype vectors have also been developed using other systems including vesicular stomatitis virus [87] and HIV [97].

Inducers of an inhospitable host

Filoviruses have a host range that appears to be restricted to humans and NHP. Rodents are not susceptible to filoviral-induced disease unless specifically adapted strains are used [16,17,86]. However, IFN-deficient mouse systems are susceptible to wild-type Ebola virus strains on first exposure [86]. Pretreating cells with type I IFN can inhibit viral replication, and various mouse studies suggest that IFN can be effective. IFNs function by activating cellular defense mechanisms. These mechanisms are nonspecific.

IFN-α_{2b} was not successful as a postexposure treatment for Ebola HF in NHP [93]. Likewise, IFN-α_{2a} showed little efficacy when administered to baboons shortly before and after Ebola virus challenge [94] or to African green monkeys when administered shortly after Marburg virus challenge [98]. However, recent studies with other viruses have suggested that antiviral activities may depend on the species of IFN used. Further studies with different IFNs have yet to be performed. An alternative strategy is to induce the host innate immune response through chemical or molecular inducers. Polyriboinosinic/polyribocytidylic acid (poly IC/LC) was shown to protect mice from lethal Ebola virus challenge [99]. It is possible that these compounds induce activation of the RNA-dependent protein kinase (PKR), 2´-5´ oligoadenylate synthetase (OAS) and Mx systems. Activation of these systems renders cells retractile to infection. Other small molecules could be used to activate the toll-like receptor (TLR) to stimulate the system with a similar activity as that of poly IC/LC [100].

Compounds that mitigate the impacts of the disease

Filoviruses are highly aggressive pathogens that cause a rapid loss of homeostasis in the infected host. One strategy to treat these acute infections and improve survival is to modulate the host immune response. To date, there are few reports describing efforts to alter the disordered proinflammatory response that seems to be an important attribute of filoviral HF. Partial protection of small cohorts of Marburg virus-infected guinea-pigs was demonstrated using the immunodulator desferal [73], or with IL-1 receptor antagonist (IL-1RA) or anti-TNF-α serum [101]. In view of the potentially important pathogenic role of TNF-α and other cytokines such as IL-6 in filoviral HF, the *in vivo* neutralization of IL-6 could have therapeutic utility in ameliorating the lethal effects of Ebola virus HF. Anti-IL-6 approaches can mitigate the deleterious effects of endotoxin-induced sepsis in several animal models [102,103].

Apoptosis of bystander lymphocytes is another important feature of filoviral HF, and ostensibly contributes to overt immunosuppression. Therapeutic interventions to protect lymphocytes have been employed for other diseases. As an example, treating HIV-infected mice with a neutralizing anti-TNF-related apoptosis-inducing ligand (TRAIL) monoclonal antibody significantly reduced the development of apoptotic CD4 T cells [104]. In addition, caspase inhibitors showed some protection of lymphocytes and improvement in survival in murine models of sepsis [105]. Clearly, treatments directed at protecting bystander lymphocytes from premature death have merit; however, as the exact mechanism(s) responsible for inducing lymphocyte apoptosis during filoviral HF have yet to be elucidated, the development of specific protection strategies will be problematic. Moreover, in order for such strategies to have utility for filoviral infections, it will likely be necessary to restrict protection from apoptosis to lymphocyte populations and not to filovirus-infected cells, which ideally would be eradicated and not inadvertently spared.

A final host-modulation strategy that may have utility in combating filoviral infections is to treat the coagulation disorders that develop during the course of disease. Although heparin appeared to have some benefit as a supportive treatment in two patients infected with Marburg virus [106], it did not improve outcome in treating Ebola HF [48]. As noted previously, infection of primate monocytes/macrophages induces the production of tissue factor [14], and a treatment strategy was based on this finding. Indeed, in a proof-of-concept study, a third of Ebola virus-infected rhesus monkeys that were treated with a protein, recombinant nematode anticoagulant protein c2 (rNAPc2), that prevents blood clotting by blocking the Factor VIIa/tissue factor pathway, survived challenge in a uniformly lethal model [26].

Other modulators of blood coagulation may also have use in treating filoviral HF. For example, temporal analysis of Ebola-infected NHP suggests that substantial declines in circulating levels of plasma protein C may in part contribute to the coagulopathy that typifies filoviral HF [14]. It is worthy to note that

recombinant human activated protein C can significantly reduce mortality in patients with severe sepsis [107], and may also have use in treating cases of filoviral HF.

Conclusion

Filovirus drug discovery is at a crossroads. The first compounds are beginning to show some efficacy in NHP models. Several classes of compounds are being developed. This review of findings from a number of studies suggests that combinations of antivirals may be needed to achieve sufficient efficacy. For example, a compound inhibiting viral replication and a separate compound modifying disease may be needed. Several viruses require combination therapy to be effective; HIV and hepatits C virus. There is a paucity of compounds that are ready for advanced development. Much of the basic understanding of filoviruses and the molecular mechanisms by which they cause disease remains lacking, and therein lays the difficulty in developing novel targets.

Expert commentary

Novel inhibitors of filoviruses are winding their way through *in vitro* systems and early *in vivo* efforts to demonstrate efficacy. However, it is likely that there will be no 'magic bullet' that will be able to confer uniform postexposure protection against these highly aggressive pathogens. Rather, combinations of drugs may be required. The critical factor in advancing promising antiviral strategies is the identification of a pharmaceutical partner to invest opportunity costs into the movement of compounds from the laboratory environment to the market. Historically, there has been a small global market for treatments and therapies against rare and exotic pathogens, such as the filoviruses. Government efforts have been made to generate artificial markets; however, these are minuscule in comparison with traditional pharmaceutical areas. Industrial partners bring unique skill sets, specifically, in bringing compounds through the regulatory and approval process. It is essential that promising compounds be partnered with a pharmaceutical company.

Five-year view

The availability of plasmid-based systems for Ebola and Marburg viruses, and the relatively recent development of reverse genetics systems for Ebola virus will continue to allow investigators to dissect critical molecular actions of these viruses in the host system. During the next 5 years, we expect that these systems will be exploited to identify important drugable targets. New fluorescent cell-based drug-discovery assays will facilitate high-throughput screening of chemical libraries, and should be pivotal in identifying candidate antivirals. Moreover, advances in the development of efficient delivery systems for antigene technologies, such as RNAi, will make this a viable approach for filoviral therapeutics. Finally, we believe that uniform postexposure protection in the more stringent NHP models is achievable, but will depend on the prompt initiation of treatment, and will probably require combinations of different drugs.

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Key issues

- Infection with Ebola or Marburg virus is usually fatal. However, outbreaks of Ebola and Marburg hemorrhagic fever (HF) are infrequent and have been geographically restricted primarily to Central Africa.
- There are no vaccines or treatments available for filoviral HF. Significant advances have been made in developing candidate vaccines against Ebola and Marburg viruses; however, the development of therapeutics lags far behind.
- A recent and highly publicized outbreak of Marburg virus in Angola coupled with the increased awareness of bioterrorism has dramatically changed perspectives regarding the need for therapeutics against Ebola and Marburg viruses.
- The efficacy of any treatments or therapies against Ebola or Marburg viruses cannot practically or ethically be assessed in humans. Approval of therapeutics for use in humans by the US FDA will probably rely on a bypass rule that permits companies to use preclinical test data demonstrating efficacy in two relevant animal models in conjunction with Phase I studies.
- A number of promising technologies, including antigene strategies and drugs that modulate coagulation disorders that typify filoviral HF, are under development as potential treatments. However, results from studies of animal models suggest that effective treatment may require a multipronged approach that employs several compounds targeting different pathways.

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